RESEARCH NOTE

Emergence of ciprofloxacin resistance in *Escherichia coli* isolates from outpatient urine samples

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ABSTRACT

This study investigated the association between prescription of fluoroquinolones and emergence of ciprofloxacin resistance among *Escherichia coli* isolates in the urine of outpatients from whom a ciprofloxacin-sensitive *E. coli* strain had been isolated previously. Patients were identified and followed using the healthcare databases of Emilia-Romagna Region, Italy. The outcome of interest was the first isolation from urine of an *E. coli* strain resistant to ciprofloxacin. Prescription of fluoroquinolones during the previous 6 months was associated independently with the emergence of ciprofloxacin resistance; the strength of the association varied according to individual fluoroquinolone agents.

Keywords Ciprofloxacin, emergence of resistance, *Escherichia coli*, fluoroquinolones, outpatients, urinary tract infection

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The prevalence of resistance to fluoroquinolones among *Escherichia coli* isolates from urine in Emilia-Romagna (a northern region of Italy with 4 million inhabitants) was 16.4% in 2003 and 17.2% in 2004 [1]. Human consumption of fluoroquinolones in the community in Emilia-Romagna was 2.3 defined daily doses (DDDs)/1000 inhabitant-days in 2003 and 2.2 DDDs/1000 inhabitant-days in 2004; the agents prescribed most frequently in 2004 were levofloxacin, ciprofloxacin, norfloxacin and moxifloxacin (39%, 30%, 11% and 11% of total fluoroquinolone DDDs, respectively) (unpublished data). The aim of the present study was to investigate the association between prescription of fluoroquinolones and emergence of ciprofloxacin resistance among *E. coli* isolates in the urine of outpatients from whom a ciprofloxacin-sensitive *E. coli* strain had been isolated previously.

Data for the study were obtained from the antimicrobial resistance surveillance system of Emilia-Romagna, as well as databases concerning patterns of care in the resident population, home care and mortality. Criteria for inclusion of patients in the study were: age ≥18 years; residence in Emilia-Romagna; one or more urine cultures positive for *E. coli* during 2003–2004, with antibiotic susceptibility data available; and a first (or only) *E. coli* isolate that was sensitive to ciprofloxacin, and that grew from an outpatient urine sample. The outcome of interest was the first isolation of a ciprofloxacin-resistant *E. coli* from an outpatient urine sample. *E. coli* isolates with ciprofloxacin MICs ≤1 mg/L, or ciprofloxacin zone diameters ≥21 mm with a 5-μg disk, were defined as susceptible, while strains with ciprofloxacin MICs ≥4 mg/L, or ciprofloxacin zone diameters ≤15 mm, were defined as resistant [2]. The date of entry into the cohort was the date on which the first sample (ciprofloxacin-susceptible) was received. Patients were followed until 31 December 2004; follow-up ended with the first isolation of a ciprofloxacin-resistant *E. coli* isolate, death, admission to hospital, or referral to the home care service.

Using the cohort as a base, all subjects were included in a case-control study and were considered as cases if a ciprofloxacin-resistant *E. coli* strain had been isolated during follow-up. The date of selection for the case-control study corresponded with the day of exit from the cohort or with the end of follow-up. All systemic antibiotics (J01 Anatomic Therapeutical Chemical Group (ATC); http://www.whocc.no/ateddd/) prescribed during follow-up were considered and grouped, according to the ATC, as fluoroquinolones (further catagorised as ciprofloxacin,

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norfloxacin, levofloxacin and other fluoroquinolones), other quinolones, cephalosporins, extended-spectrum penicillins, penicillins/β-lactamase inhibitors, macrolides, nitrofurantoin, fosfomycin, trimethoprim–sulphamethoxazole, tetracyclines, aminoglycosides, or other agents. Exposure to one group of antibiotics was categorised according to the period between the date of selection for the case-control study and the date of the last prescription of an agent belonging to that group (patients who were prescribed an agent within 180 days before the date of selection were regarded as exposed). Other variables considered were gender, age at time of selection for the case-control study, duration of follow-up, number of relapses/re-infections with non-resistant strains of *E. coli* isolated from urine during follow-up, and the local health trust of the laboratory.

STATA v.8.0 (Stata Corp., College Station, TX, USA) was used for analysis, with p 0.05 considered significant. Univariate analysis was conducted by Poisson regression. For the case-control study, categorical variables were compared using chi-square tests or Fisher exact tests, as appropriate, while Student's *t*-test was used for quantitative variables. Multivariate analysis was performed with unconditional logistic regression using the likelihood ratio test.

The study population comprised 11 428 subjects with isolation of community-acquired *E. coli* from urine. Urine cultures were processed by ten laboratories in Emilia-Romagna that belonged to eight of the 16 local health trusts of the region. The overall isolation rate of ciprofloxacin-resistant *E. coli* was 23/1000 person-years, but was higher for older individuals and for those with relapses/re-infections caused by non-resistant strains during follow-up. Local health trusts were associated significantly with the outcome, but no association was observed with the calendar period (semesters of follow-up).

ORs for isolation of resistant *E. coli* decreased, with an increasing period between the last prescription of fluoroquinolones and the date of selection for the case-control study (Fig. 1). In the multivariate analysis, prescription of fluoroquinolones during the previous 180 days was associated significantly with isolation of resistant *E. coli* from urine; the strength of the association varied according to the individual agents (the highest OR was observed for ciprofloxacin), suggesting a different potential for selection of resistance. Other independent variables associated with the outcome were prescription of penicillins/β-lactamase inhibitors or cephalosporins, relapses/re-infections caused by non-resistant strains during the follow-up, older age categories, duration of follow-up, female gender, and the local health trust of the laboratory (Table 1). The associations between prescription of penicillins/β-lactamase inhibitors and cephalosporins, and the emergence of resistance to ciprofloxacin in *E. coli*, could be related to the observed presence of co-resistance between these agents and ciprofloxacin. The increased risk of resistance in subjects with relapses/re-infections can be explained by the measure of exposure to infection by uropathogens, including ciprofloxacin-resistant *E. coli*, which is provided by this variable.

A limitation of the study was the absence of clinical information concerning the severity of the infection and/or the presence of complicating conditions. Moreover, data concerning prescriptions from the private sector were not recorded in the database, leading to a possible underestimate of exposure to antibiotics, which could bias ORs

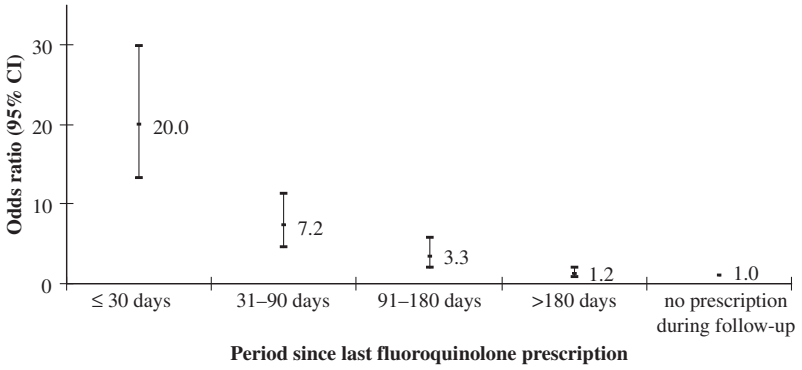

Fig. 1. Association between previous use of fluoroquinolones and emergence of ciprofloxacin resistance among *Escherichia coli* isolates from urine. Previous use of fluoroquinolones was categorised according to the time of the last prescription before the date of selection for the case-control study.

Table 1. Factors associated with the emergence of ciprofloxacin resistance among *Escherichia coli* isolates from the urine of outpatients (univariate and multivariate analyses of the case-control study)

	Cases (%)	Controls (%)	p values[a]	Adjusted ORs (95% CI)	p values[c]
Total n = 11 428	237	11 191			
Age category (years)			<0.001		<0.001
18–49	26 (11.0)	3921 (35.0)		1	
50–64	46 (19.4)	2058 (18.4)		3.0 (1.8–5.0)	
≥65	165 (69.6)	5212 (46.6)		4.0 (2.6–6.1)	
Gender			0.306		0.028
Male	25 (10.5)	1431 (12.8)		1	
Female	212 (89.5)	9760 (87.2)		1.6 (1.0–2.5)	
No. of relapses/re-infections			<0.001		<0.001
0	158 (66.7)	9471 (84.6)		1	
1	49 (20.7)	1226 (11.0)		2.7 (1.9–3.8)	
≥2	30 (12.7)	494 (4.4)		4.6 (2.8–7.3)	
Days of follow-up (mean)	195.7	329.8	<0.001[d]	0.996[b]	<0.001
Fluoroquinolones			<0.001		<0.001
Ciprofloxacin	116 (49.0)	1502 (13.4)		6.2 (4.4–8.7)	
Norfloxacin	33 (13.9)	574 (5.1)		4.5 (2.8–7.1)	
Levofloxacin	21 (8.9)	659 (5.9)		2.5 (1.5–4.2)	
Other fluoroquinolones	8 (3.4)	226 (2.0)		3.1 (1.5–6.8)	
Other quinolones	5 (2.1)	92 (0.8)	0.051[e]		
Fosfomycin	13 (5.5)	866 (7.7)	0.198		
Penicillins/β-lactamase inhibitors	39 (16.5)	1005 (9.0)	<0.001	1.8 (1.2–2.7)	0.003
Extended-spectrum penicillins	22 (9.3)	730 (6.5)	0.090		
Cephalosporins	24 (10.1)	588 (5.3)	0.001	1.7 (1.1–2.7)	0.038
Macrolides	12 (5.1)	571 (5.1)	0.978		
Nitrofurantoin	0 (0)	10 (0.1)	1[e]		
Trimethoprim–sulphamethoxazole	16 (6.8)	432 (3.9)	0.023		
Aminoglycosides	3 (1.3)	35 (0.3)	0.044[e]		
Tetracyclines	0 (0)	30 (0.3)	1[e]		
Other agents	3 (1.3)	49 (0.4)	0.093[e]		

[a]Chi-square test.
[b]OR for 1-day increase of follow-up.
[c]Likelihood ratio test.
[d]Student's t-test.
[e]Fisher's exact test.

towards a value of 1. Another potential limitation, which could similarly bias the ORs, is the misclassification of a proportion of cases as controls when a microbiological diagnosis of the resistant infection was either not performed or was performed at a laboratory not included in the regional surveillance system [3]. Finally, the number of ciprofloxacin-susceptible *E. coli* isolates after entry into the cohort is only a proxy variable that probably underestimates the real frequency of relapses/re-infections, as not all urinary tract infections are cultured, or culture occurs in laboratories not included in the regional surveillance system. An underestimate of the number of relapses/re-infections could prevent full elimination of the positive confounding effect caused by the exposure, leading to an overestimate of the ORs for the association between previous prescription of fluoroquinolones and the emergence of resistance to ciprofloxacin.

Nevertheless, the study cohort was a well-defined population that was not initially infected by ciprofloxacin-resistant *E. coli*, and for which infections diagnosed during follow-up were very unlikely to be healthcare-related. The recorded data also allowed adjustment for the duration of follow-up [4]. The results showed a strong association between previous prescriptions of fluoroquinolones and the emergence of resistance to ciprofloxacin in *E. coli* from community-acquired urinary tract infections. Fluoroquinolones should thus be used prudently, avoiding unnecessary prescriptions and considering alternative regimens both for urinary tract and for respiratory tract infections. The increased risk of *E. coli* resistance to ciprofloxacin in patients with a history of recent treatment with fluoroquinolones could be considered a criterion for choosing an alternative therapy for community-acquired urinary tract infections.

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RESEARCH NOTE

Allelic polymorphism in the *Plasmodium vivax* dihydrofolate reductase gene among Indian field isolates

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ABSTRACT

In total, 129 *Plasmodium vivax* isolates from different geographical areas in India were analysed for point mutations in the *P. vivax* dihydrofolate reductase gene that were associated with pyrimethamine resistance. A gradual increase in the frequency of mutant genotypes was observed from north to south ($p < 0.0001$). In the northern region (Delhi, Panna and Nadiad), the wild-type genotype was most prevalent, while the mutant genotype predominated in the coastal regions of southern India (Navi Mumbai, Goa and Chennai). Isolates from the Car-Nicobar islands showed only mutant genotypes. The differential geographical pattern of mutations may be associated with the transmission pattern.

Keywords Allelic polymorphism, dihydrofolate reductase gene, genotypes, geographical distribution, malaria, *Plasmodium vivax*

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The worldwide spread of chloroquine-resistant strains of *Plasmodium falciparum* has led to the use of sulphadoxine–pyrimethamine as the first-line anti-malarial agent in south-east Asian countries. Sulphadoxine and pyrimethamine sequentially inhibit the dihydropteroate synthase (Dhps) and dihydrofolate reductase (Dhfr) enzymes, respectively, in the folate biosynthesis pathway to give a synergic anti-malarial effect [1]. However, *P. falciparum* has overcome the effect of sulphadoxine–pyrimethamine by evolving point mutations in the genes encoding the Dhps and Dhfr enzymes that reduce their drug-binding affinity [2,3]. In India, chloroquine remains the first-line anti-malarial agent for treatment of both *P. falciparum* and *Plasmodium vivax*. Chloroquine resistance in *P. falciparum* has been reported in India [4,5], and areas with a chloroquine resistance level of >25% have switched to the use of sulphadoxine–pyrimethamine as the first-line anti-malarial agent. Although *P. vivax* is still susceptible to chloroquine in India [6,7], the use of sulphadoxine–pyrimethamine to treat chloroquine-resistant *P. falciparum* is creating selection pressure in the *P. vivax* population. Therefore, the aim of the present study was to obtain information concerning mutations related to pyrimethamine resistance in the *P. vivax dhfr* gene of Indian field isolates.

A previous study [8] identified six new mutations in the *P. vivax dhfr* gene by sequencing, but none was located in the active sites [9]. Therefore, in order to screen field isolates for *P. vivax dhfr*

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